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Factors Influencing Discrimination between Insecticide-Treated and Untreated Foods by Northern Bobwhite

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Tests were conducted to determine at what dietary concentrations northern bobwhite (Colinus virginianus) chicks (14 days old) could discriminate between pesticide-treated and untreated food using an organophosphate (OP) insecticide, parathion, and two carbamates, carbofuran and methiocarb. Results from subacute dietary LC50 tests (one feeder of treated food per cage) were compared to tests where birds were presented with two feeders (one treated, one untreated, 1:1) or 10 feeders (five treated, five untreated, 5:5; or nine treated , one untreated, 9:1). The dietary concentration above which birds discriminate between feeders by consuming a greater proportion of untreated food is defined as the discrimination threshold (DT). The DT occurred at sublethal concentrations in all 1:1 tests, with little mortality or reduction in food consumption. Little or no discrimination was observed in 9:1 tests, with mortality similar to the LC50 tests. The discrimination response in 5:5 tests was similar to the 1:1 tests for parathion and methiocarb, but with carbofuran the DT was higher than in the 1:1 test and higher mortality was observed. In all tests, mortality was inversely related to total food consumption. No relationship was found between mortality and the amount of active ingredient ingested/bird-day. Consequently, mortality was more a function of ability to locate untreated feeders than amount of chemical ingested. When alternative food choices exist, vulnerability to poisoning can be influenced by the number and relative abundance of those choices, as well as the bird's ability to detect the chemical.

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Factors Influencing Discrimination between Insecticide-Treated and Untreated Foods by Northern Bobwhite

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Abstract. Tests were conducted to determine at what dietary concentrations northern bobwhite (*Colinus virginianus*) chicks (14 days old) could discriminate between pesticide-treated and untreated food using an organophosphate (OP) insecticide, parathion, and two carbamates, carbofuran and methiocarb. Results from subacute dietary LC50 tests (one feeder of treated food per cage) were compared to tests where birds were presented with two feeders (one treated and one untreated, 1:1) or 10 feeders (five treated and five untreated, 5:5; or nine treated and one untreated, 9:1). The dietary concentration above which birds discriminate between feeders by consuming a greater proportion of untreated food is defined as the discrimination threshold (DT). The DT occurred at sublethal concentrations in all 1:1 tests, with little mortality or reduction in food consumption. Little or no discrimination was observed in 9:1 tests, with mortality similar to the LC50 tests. The discrimination response in 5:5 tests was similar to the 1:1 tests for parathion and methiocarb, but with carbofuran the DT was higher than in the 1:1 test and higher mortality was observed. In all tests, mortality was inversely related to total food consumption. No relationship was found between mortality and the amount of active ingredient ingested/bird-day. Consequently, mortality was more a function of ability to locate untreated feeders than amount of chemical ingested. When alternative food choices exist, vulnerability to poisoning can be influenced by the number and relative abundance of those choices, as well as the bird's ability to detect the chemical.

The ability of birds to detect and avoid toxic foods when nontoxic alternatives are available has been demonstrated with several pesticides (Kenaga *et al.* 1979; Kononen *et al.* 1986, 1987; Bennett (in review)). These controlled-feeding tests presented groups of juvenile mallards (*Anas platyrhynchos*) or northern bobwhite (*Colinus virginianus*) with a free choice between two feeders—one containing untreated food and the other containing one of several dietary concentrations of test chemical. Under these conditions, birds responded to the presence of many chemicals at sublethal concentrations by decreasing the proportion of treated food consumed without significantly reducing total food consumption, thereby reducing the occurrence of toxic effects.

The field conditions under which wildlife species could utilize the ability to detect and avoid toxic foods are largely unknown. There are several factors acting in the field (*e.g.*, restricted food availability, spacial and temporal patchiness of food, physiological status, and weather) that have been removed in these laboratory tests. Bennett (in review) evaluated the effects of two factors—number of food choices available and relative availability of treated and untreated food sources—on dietary discrimination in northern bobwhite using two organophosphate insecticides. Tests with chlorpyrifos demonstrated that it was detected readily by birds at sublethal concentrations regardless of the number and relative availability of choices, whereas the response in tests with methyl parathion was affected by both the number and relative proportion of food choices. Dietary discrimination information on additional pesticides and additional bird species is needed to understand how birds react to available sensory cues and how responses are affected by changes in feeding scenarios (*i.e.*, changes in the number and relative proportion of choices). The ultimate goal of these

studies was to understand the extent to which behavioral responses of wildlife can modify dietary exposure to chemicals in the field.

The objective of the present study was to further evaluate the role of these factors—number of feeders present and the relative proportion of treated and untreated feeders—on dietary discrimination behavior in bobwhite using three cholinesterase-inhibiting insecticides. A series of feeding tests involving different combinations of treated and untreated food choices was conducted to measure the discrimination response using parathion [*O,O*-diethyl *O-p*-nitrophenyl phosphorothioate; technical purity = 98.5%], carbofuran [2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate; technical purity = 98.8%], and methiocarb [3,5-dimethyl-4-(methylthio)phenol methylcarbamate; technical purity = 97.7%].

Experimental Methods

Northern bobwhite chicks were tested in one of four dietary tests consisting of combinations of chemically-treated and untreated food: 1) one feeder/cage containing treated food (dietary LC50 test); 2) two feeders/cage, one containing treated food and one untreated (1:1); 3) 10 feeders/cage, five treated and five untreated (5:5); and 4) 10 feeders/cage, nine treated and one untreated (9:1). At 10 days of age, birds were weighed and assigned to test cages using a stratified random procedure based on body weight (Steel and Torrie 1960). At 14 days of age, birds were weighed and presented with test diets for 5 days. In each of the tests, cages were assigned randomly to one of six dietary concentrations or a carrier control group. In the 5:5 and 9:1 tests all feeders within a cage contained the same dietary concentration.

All insecticides were dissolved in corn oil in a 65°C water bath. The corn oil solution was mixed 2:98 (w/w) with Purina® gamebird starter diet for 10 min. Control diets contained corn oil in the same proportion.

Birds were housed in brooder cages measuring 70 × 91 × 22 cm, and maintained at 35°C with a 12 hr light:12 hr dark cycle. LC50 and 1:1 tests used rectangular galvanized steel chick feeders, while 5:5 and 9:1 tests used 500-ml rectangular polyethylene bottles with four 2.5-cm holes in the sides for feeding. Bottle feeders were arranged in two rows of five aligned from front to back in each brooder and held in place by metal frames. Since birds can quickly develop preferences among feeder positions, feeders were moved daily to reduce the association of toxic effects with a particular location and to force birds to respond to stimuli provided by the food. The position of feeders in the 1:1 test was changed on a random schedule. In order to ameliorate the effects of potential feeder position bias in the 5:5 and 9.1 tests, each bottle feeder spent equal time in each of the five positions in the tray during the 5-day treatment period, using a stratified random schedule. Food and water were provided *ad libitum*, and each individual feeder contained more than the total amount of feed consumed daily by the control groups.

Food consumption, clinical signs of toxicity, and mortality were recorded daily in the LC50 test during the 5-day treatment and 3-day post-treatment periods. Food consumption (corrected for spillage) was calculated as grams consumed per bird-day, where bird-day equaled the average number of birds alive at the beginning and end of each 24-hr period. Individual body weights were recorded on days 0, 5, and 8. The same measurements were taken in the food choice tests, except that all surviving birds were asphyxiated with CO_2 at the end of the 5-day treatment period.

In the LC50 tests, the median lethal concentration (LC50) and 95% confidence interval were calculated by probit analysis using the Statistical Analysis System computer software package (SAS Institute Inc 1982). In the food choice tests, the dietary concentration above which birds discriminated between treated and untreated foods by consuming a higher proportion of untreated food was defined as the discrimination threshold (DT). The DT was calculated as the intersection of a two-phase regression using the log of the ratio of untreated to treated food consumption over the 5-day test period as the dependent variable and log of concentration of treated food as the independent variable (Bennett and Schafer, in press). At concentrations below the DT the expected value of the response, y = log (untreated/treated), should equal 0, reflecting no preference for either food choice. At higher concentrations the regression has a positive slope, indicating a dose-related increase in the probability of selecting untreated food. In the 9:1 tests, the dependent variable was modified to y = log ((untreated/treated)/(1/9)), to account for the change in relative availability of the two food types so that the expected value for y equaled 0 at concentrations below the DT. This method was based on the assumption that the two-phase regression is an appropriate model.

Food consumption and body weight data were analyzed by fitting linear regression models to various dose ranges and selecting the best fit by using the criterion of the highest coefficient of determination. Dietary concentrations were transformed to log (concentration + 1). Regression slopes are presented ± one standard error.

Results

Parathion

The LC50 test with parathion produced an LC50 value (95% confidence interval) of 177 ppm or mg/kg (154, 212). Hill *et al.* (1975) calculated an LC50 of 194 ppm for the same age bobwhite. Mean body weights of control birds increased 60% during the 5-day treatment period, while decreasing in all dietary concentrations that resulted in one or more deaths (Table 1). The relationship of dietary concentration (x) to percent weight change (y) was $y = 2.43 - (1.19 \pm 0.16) \log(x + 1)$, $79 < x < 251$, $R^2 = 0.93$. All bird mortality occurred between days 3 and 6, with an average loss of 37% ± 1 (SE) of their body weight during treatment (range: 21–43% loss). Total food consumption was reduced in all treated groups and generally decreased with increasing dietary concentration [$y = 15.8 - (5.9 \pm 1.4) \log(x + 1)$, $79 < x < 251$, $R^2 = 0.80$] (Figure 1). The greatest ingestion of active ingredient occurred in the 126 ppm group, the lowest concentration producing mortality, with chemical ingestion decreasing thereafter (Table 1).

Table 1. Percent mortality, dietary exposure, and weight changes for bobwhite chicks presented one feeder (LC50) or various combinations of feeders containing *parathion*-treated or untreated food during a 5-day treatment period

Test (TRT:UNT)	Dietary conc. (ppm)	N	% Mortality (Day 0–5)	Food (g/b–d)		Chemical ingestion (mg/kg/d)	% Body weight change (Day 0–5)	
				UNT	TRT		$\bar{x}$	Range
LC50	0	10	0	7.2	0	0	60	54,78
	79	10	0	0	4.2	10.54	15	−10,31
	100	10	0	0	3.8	12.12	12	−9,41
	126	10	20	0	4.5	18.02	−8	−36,11
	159	10	50	0	2.9	14.60	−29	−41,−1
	200	10	60	0	2.0	12.69	−32	−42,−13
	251	10	80	0	1.5	11.67	−38	−43,−28
1:1	0	10	0	7.2	0	0	60	64,78
	50	10	0	4.1	2.9	4.49	46	28,53
	79	10	0	5.1	2.0	4.76	48	41,58
	100	10	0	4.8	1.9	5.76	48	38,54
	126	10	0	3.4	2.1	8.21	29	13,48
	159	10	0	5.0	1.7	8.90	44	26,52
	200	10	0	5.4	0.9	5.90	43	21,53
5:5	0	10	0	6.8	0	0	52	49,55
	50	10	0	3.8	3.1	5.04	55	47,62
	79	10	0	3.6	2.7	6.83	46	18,56
	100	10	0	4.4	1.6	5.13	46	40,53
	126	10	0	3.1	2.3	9.09	35	−9,56
	159	10	0	3.8	1.6	8.12	37	24,51
	200	10	0	4.3	1.4	8.92	41	25,55
9:1	0	10	0	7.1	0	0	50	42,58
	50	10	10	1.0	4.8	6.67	28	−13,57
	79	10	0	1.5	4.2	9.28	31	15,41
	100	10	0	0.8	3.6	10.45	12	−4,24
	126	10	0	1.0	3.1	11.00	6	−14,29
	159	10	30	0.1	2.2	10.78	−27	−41,3
	200	10	50	0.2	1.8	10.05	−34	−45,−12

The responses in the 1:1 and 5:5 tests were similar. No mortality or signs of intoxication were observed in any treatment group. The DTs were 33 and 38 ppm for the 1:1 and 5:5 tests, respectively, and were below dietary concentrations producing mortalities in the LC50 test. The calculated Effective Avoidance Indexes (EAI), LC50/DT, for these tests were 5.4 and 4.7, respectively. Little or no reduction in total food consumption was observed from control to treated groups because increased consumption of untreated food compensated for reductions in consumption of treated food (Figure 1). Ingestion of active ingredient in the 1:1 and 5:5 tests was greatest at approximately the same concentration as in the LC50 (126 ppm), but at approximately half the amount ingested in the LC50 (Table 1). Although body weight gains were lower than for control birds, all treated groups gained ≥29% during treatment. The slopes of the dose-weight change relationships were significantly different ($P < 0.05$) from the LC50 test for the 1:1 test [$y = 0.66 - (0.12 \pm 0.16) \log(x + 1)$, $50 < x < 200$, $R^2 = 0.12$] and 5:5 test [$y = 1.02 - (0.29 \pm 0.09) \log(x + 1)$, $50 < x < 200$, $R^2 = 0.70$].

In the 9:1 test, birds did not discriminate between feeders and no DT could be established. Consequently, the responses in this test were similar to the LC50. There was a dose-related reduction in total food consumption [$y = 18.6 - (7.1 \pm 1.3) \log(x + 1)$, $50 < x < 200$, $R^2 = 0.89$], with all groups consuming 73 to 94% treated food. Dose-related changes in body weight also were not significantly different ($P > 0.05$) than in the LC50 test [$y = 2.41 - (1.17 \pm 0.26) \log(x + 1)$, $50 < x < 200$, $R^2 = 0.84$] (Table 1).

Carbofuran

The calculated LC50 value (95% confidence interval) for carbofuran was 1,144 ppm (873, 1605). Two types of mortality were observed in this test:

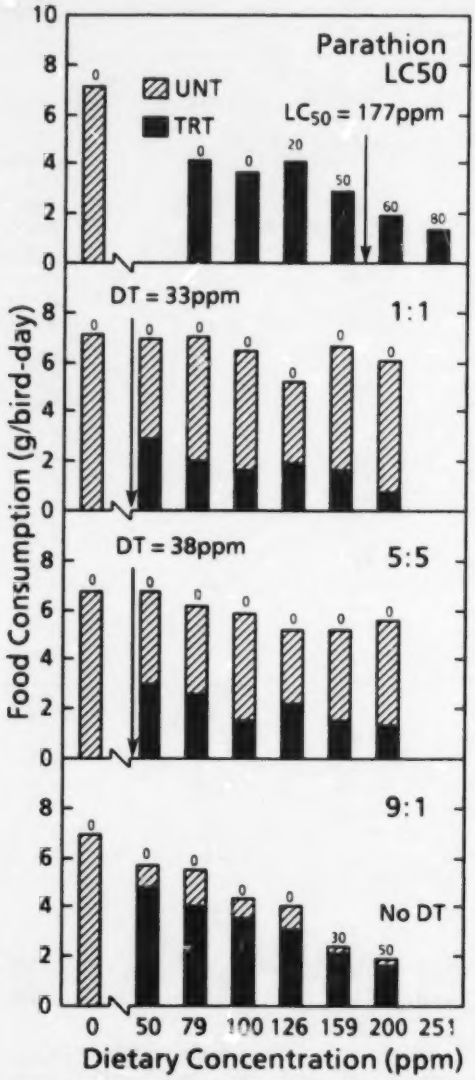

Fig. 1. Average daily food consumption for northern bobwhite presented various combinations of parathion-treated and untreated feeders. The values above each bar represent percent mortality during the 5-day treatment period. The discrimination threshold (DT) represents the dietary concentration above which birds show a preference for untreated food

1) acute poisoning within 1 day, with little or no weight loss ($x = -5\%$; $n = 6$) and 2) death occurring 3 to 6 days from test initiation, with significant weight loss ($x = -38\% \pm 1$ (SE); $n = 16$). The relationship of dietary concentration to percent weight change was $y = 2.06 - (0.71 \pm 0.19) \log(x + 1)$, $520 < x < 2000$, $R^2 = 0.82$. There also was a dose-related reduction in total food consumption [$y = 19.7 - (5.3 \pm 1.7) \log(x + 1)$, $520 < x < 2000$, $R^2 = 0.86$] (Figure 2). The greatest chemical ingestion occurred at the highest dietary concentration tested (Table 2).

While the DTs were 170 and 400 ppm for the 1:1 and 5:5 tests, respectively, and birds were clearly able to discriminate between treated and untreated feeders after the first day, there were several day 1 mortalities from acute poisoning in these tests. Additionally, there were three deaths in the 5:5 test occurring on days 4 and 5 with weight changes of -45 to $+16\%$ from the start of treatment. There also were treatment effects on body weight changes at the higher doses in the 1:1 test [$y = 1.65 - (0.42 \pm 0.08) \log(x + 1)$, $520 < x < 2000$, $R^2 = 0.91$] and 5:5 test [$y = 2.09 - (0.61 \pm 0.30) \log(x + 1)$, $520 < x < 2000$, $R^2 = 0.58$] (Table 2). Little or no reduction in total food consumption was observed from control to treated groups in the 1:1 test and small reductions were observed in the higher concentrations of the 5:5 test (Figure 2). The EAI was 6.7 for the 1:1 test, which is comparable to 5.8 reported for bobwhite by Kononen *et al.* (1987), but dropped to 2.9 for the 5:5 test. Chemical ingestion was greatest at the highest dietary concentration tested in both tests (Table 2).

In the 9:1 test, birds did not discriminate between feeders and no DT could be established. There was a dose-related reduction in total food consumption [$y = 20.1 - (5.2 \pm 1.3) \log(x + 1)$, $520 < x < 2000$, $R^2 = 0.85$], with all groups consuming 69 to 84% treated food and the greatest chemical ingestion occurring at 2000 ppm. Dose-related changes in body weight also were not significantly different ($P > 0.05$) than in the LC50 test [$y = 3.40 - (1.11 \pm 0.16) \log(x + 1)$, $520 < x < 2000$, $R^2 = 0.94$]. Of the 11 birds that died in this test, four died within the first day with only a 1 to 3% loss in body weight and seven died on days 3 and 4 with an average 39% weight loss (Table 2).

Methiocarb

The LC50 test with methiocarb produced an LC50 value (95% confidence interval) of 1,457 ppm $(0,\infty)$. The poor fit of the probit regression resulted from a single mortality in the 501 ppm group (Table 3). Mean body weights of controls increased 53% during the 5-day treatment period, while decreasing in dietary concentrations that resulted in >10% mortality (Table 1). The relationship of dietary concentration to percent weight change was $y = 2.57 - (0.86 \pm 0.16) \log(x + 1)$, $200 < x < 2000$, $R^2 = 0.87$. All bird mortality occurred between days 3

Table 2. Percent mortality, dietary exposure, and weight changes for bobwhite chicks presented one feeder (LC50) or various combinations of feeders containing *carbofuran*-treated or untreated food during a 5-day treatment period

Test (TRT:UNT)	Dietary conc. (ppm)	N	% Mortality (Day 0–5)	Food (g/b–d)		Chemical ingestion (mg/kg/d)	% Body weight change (Day 0–5)	
				UNT	TRT		$\bar{x}$	Range
LC50	0	10	0	6.8	0	0	49	36,61
	520	10	20	0	5.6	94.0	16	−44,48
	729	10	20	0	4.2	98.7	1	−25,43
	1020	10	30	0	3.6	123.8	−9	−45,17
	1428	10	60	0	2.2	101.5	−31	−45,−12
	2000	10	90	0	2.7	179.5	−21	−45,−1
1:1	0	10	0	6.8	0	0	49	36,61
	520	10	0	4.1	2.3	38.9	48	41,59
	729	10	0	4.6	2.1	50.3	48	22,60
	1020	10	0	5.2	1.2	41.1	43	28,51
	1428	10	10	5.4	1.0	48.9	33	−3,53
	2000	10	20	4.6	1.4	90.9	25	1,46
5:5	0	10	0	7.0	0	0	51	42,60
	520	10	10	4.2	3.0	49.6	42	16,53
	729	10	0	3.5	2.6	59.4	38	6,55
	1020	10	10	2.8	2.4	78.6	11	−3,28
	1428	10	10	4.4	1.8	82.7	34	−3,47
	2000	10	50	3.5	1.3	87.5	−1	−45,24
9:1	0	10	0	7.0	0	0	51	42,60
	520	10	0	1.3	4.4	75.6	33	9,55
	729	10	0	0.9	4.9	118.6	26	2,43
	1020	10	20	1.3	3.0	99.3	4	−37,20
	1428	10	40	0.5	2.5	115.4	−23	−44,−2
	2000	10	50	0.9	2.4	162.9	−24	−45,−2

and 6, with an average loss of 40% ± 1 (SE) of their body weight during treatment (range: 35–47% loss). Total food consumption decreased with increasing dietary concentration [$y = 18.6 - (4.9 \pm 1.1) \log(x + 1)$, $200 < x < 2000$, $R^2 = 0.83$] (Fig. 3). The greatest ingestion of active ingredient occurred in the 1,259 ppm group (20% mortality), but decreased by half in the 2,000 ppm group (90% mortality).

The DTs for the 1:1 and 5:5 tests essentially were equal (224 and 222 ppm, respectively) and were lower than concentrations producing mortalities in the LC50 test. The EAI for both tests was 6.5, which is lower than the 8.7 calculated for bobwhite by Kononen *et al.* (1986). There was little or no treatment effect on total food consumption in the 1:1 test (Figure 3), but a dose-related decrease was observed in the 5:5 test [$y = 11.4 - (1.8 \pm 0.4) \log(x + 1)$, $200 < x < 2000$, $R^2 = 0.80$]. Chemical ingestion was greatest at 2,000 ppm in both tests, after being relatively similar at the lower concentrations. Only one death was recorded in these tests: the smallest bird (day 0 weight = 20 g, compared to a mean weight = 32 g for all birds) used in the test died on day 3 in the 2,000 ppm group of the 1:1 test. The slopes of the dose-weight change relationships were significantly different ($P < 0.05$) from the LC50 test for the 1:1 test [$y = 0.76 - (0.11 \pm 0.04) \log(x + 1)$, $200 < x < 2000$, $R^2 = 0.68$] and 5:5 test [$y = 1.13 - (0.27 \pm 0.04) \log(x + 1)$, $200 < x < 2000$, $R^2 = 0.91$].

There was little or no discrimination between feeders in the 9:1 test, except at the highest concentration (2,000 ppm) where 42% of all food consumed was untreated (Figure 3). Although there was a tendency to discriminate against treated food, chemical ingestion was greatest in the 2,000 ppm group (Table 3). There was a dose-related decrease in total food consumption [$y = 14.0 - (3.1 \pm 0.8) \log(x + 1)$, $200 < x < 2000$, $R^2 = 0.78$]. One bird died on day 1 with a 2% loss in body weight, and four died between days 3 and 5 with an average 37% loss. The slope of the dose-weight change relationships was not significantly different ($P > 0.05$) from the LC50 test [$y = 1.83 - (0.61 \pm 0.12) \log(x + 1)$, $200 < x < 2000$, $R^2 = 0.87$].

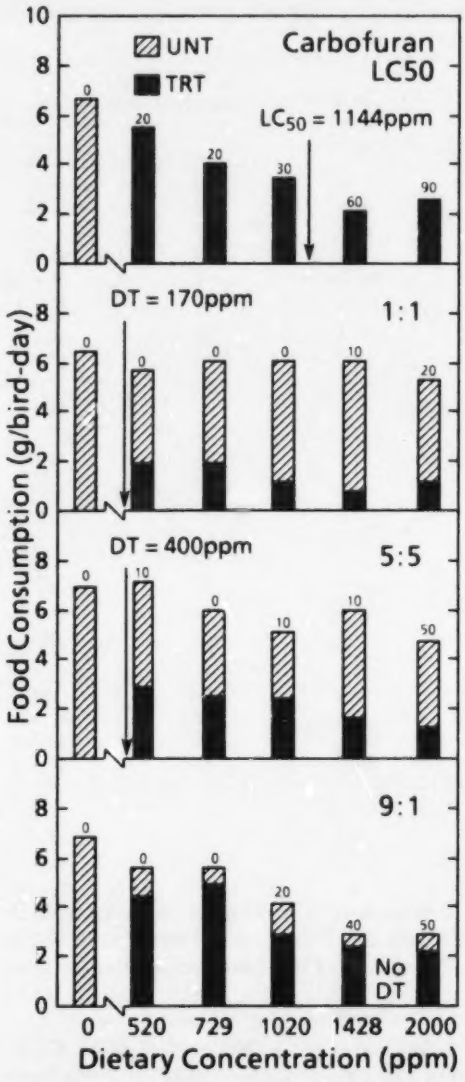

Fig. 2. Average daily food consumption for northern bobwhite presented various combinations of carbofuran-treated and untreated feeders. The values above each bar represent percent mortality during the 5-day treatment period. The discrimination threshold (DT) represents the dietary concentration above which birds show a preference for untreated food

Influences on Mortality

Because consumption of treated food generally decreased with increasing dietary concentrations, whether birds were discriminating between treated and untreated feeders or not, the amount of chemical ingestion did not increase at the same rate as the dietary concentration. In many tests there was little or no increase in chemical ingestion with increasing concentrations. Consequently, no relationship was observed between percent mortality and the amount of chemical ingestion for any of the three insecticides. However, percent mortality was inversely related to total food consumption. When the mean values of each concentration were pooled for the four tests with each chemical, the slopes of the relationship of percent mortality (y) to total food consumption (x) was significantly different ($P < 0.05$) from 0 for both parathion [$y = 109 - (26 \pm 4)\,x$, $R^2 = 0.88$] and methiocarb [$y = 120 - (25 \pm 3)\,x$, $R^2 = 0.97$]. Although 39% of all mortalities related to carbofuran occurred within the first day and were not associated with large losses of body weight, the slope of the regression model for carbofuran [$y = 95 - (14 \pm 2)x$, $R^2 = 0.74$] was significantly different ($P < 0.05$) than both 0 and the other insecticides.

Discussion

Increasing the number of feeders affected DTs only in tests with carbofuran, reducing the EAI from 6.7 to 2.9 in the 1:1 and 5:5 tests, respectively. The number of feeders had little effect on feeding responses in parathion and methiocarb tests. In contrast, the relative proportion of treated and untreated feeders (5:5 *vs* 9:1) had a significant effect on the feeding responses of bobwhite chicks. In 5:5 tests, the DTs occurred at dietary concentrations that were predicted to cause little or no mortality in the LC50 test. No mortality and little reduction in total food consumption was observed in 5:5 tests with parathion and methiocarb. Although mortality was 50% in the highest concentration of the carbofuran 5:5 test, birds preferentially fed on untreated food in every treatment group. In contrast, there was little or no discrimination between feeders in the 9:1 tests, and mortality approached levels observed in the LC50 tests, even though untreated food was available in *ad libitum* quantities.

Differences in response between chemicals are a function of the intensity of the sensory cues available to the bird from the treated food and the use of those cues in the development of conditioned aversions. Conditioned aversions usually develop when an animal associates a food, water, or place with an illness or other negative reinforcer leading to an avoidance of that item in subsequent encounters (Conover 1984). If a contaminated food is associated with a detectable sensory cue, animals form aversions to that cue, but not necessarily to the food type if uncontaminated food is available. If chemical cues are weak or undetectable, consump-

Table 3. Percent mortality, dietary exposure, and weight changes for bobwhite chicks presented one feeder (LC50) or various combinations of feeders containing *methiocarb*-treated or untreated food during a 5-day treatment period

Test (TRT:UNT)	Dietary conc. (ppm)	N	% Mortality (Day 0–5)	Food (g/b–d)		Chemical ingestion (mg/kg/d)	% Body weight change (Day 0–5)	
				UNT	TRT		$\bar{x}$	Range
LC50	0	10	0	7.0	0	0	53	39,65
	200	10	0	0	6.6	41.0	49	23,65
	316	10	0	0	6.4	65.6	47	39,57
	501	10	10	0	5.8	90.1	22	−41,42
	794	10	0	0	5.4	136.3	27	2,40
	1259	10	20	0	4.0	161.3	−4	−36,29
	2000	10	90	0	1.3	85.8	−41	−47,−37
1:1	0	10	0	7.0	0	0	53	39,65
	200	10	0	2.9	3.5	23.2	49	41,57
	316	10	0	3.9	2.7	27.5	50	42,60
	501	10	0	4.2	2.4	38.4	46	34,53
	794	10	0	5.4	1.1	30.0	48	37,59
	1259	10	0	6.7	0.7	28.3	45	17,54
	2000	10	10	5.3	0.9	60.0	37	−35,52
5:5	0	10	0	7.2	0	0	47	38,55
	200	10	0	3.2	3.9	24.6	50	41,63
	316	10	0	3.8	2.7	27.1	42	35,49
	501	10	0	5.7	1.3	21.0	44	37,61
	794	10	0	5.2	1.2	30.0	36	22,53
	1259	10	0	3.8	1.7	64.4	30	18,43
	2000	10	0	3.8	1.5	94.6	20	10,39
9:1	0	10	0	7.2	0	0	47	38,55
	200	10	0	1.2	4.9	31.4	39	32,45
	316	10	0	0.8	6.2	58.6	38	26,45
	501	10	0	2.2	3.7	58.3	24	4,35
	794	10	10	0.9	3.7	88.6	−1	−41,25
	1259	10	20	0.7	2.9	114.4	−17	−43,5
	2000	10	20	1.7	2.3	147.6	−8	−39,9

tion of both contaminated and uncontaminated food of that type may be reduced.

However, in these tests there were also two types of response to each chemical that were related to the complexity of the tests, even though the sensory cues on treated food presumably remained the same. The major difference between the 1:1 and 9:1 tests was that more sampling of feeders occurred to evaluate their contents when more feeders were present. With additional sampling, there was additional consumption of active ingredient. In the first type of response, most often observed in the 1:1 tests, birds responded to the feeders presented by readily choosing between feeders without reducing total food consumption. Little mortality, weight loss, or signs of poisoning were observed. The second type of response was most often observed in the 9:1 tests and the higher concentrations of the 5:5 tests. The higher amount of chemical ingestion associated with increased sampling caused a reduction in consumption of both treated and untreated food before a preference for untreated feeders developed. When total food consumption was reduced, little or no discrimination was observed. It is not known if the reduced consumption was due to illness or some other physiological response.

In the LC50 tests, food consumption also declined with increasing dietary concentration, consequently, the amounts of chemical consumed did not increase proportional to the concentration. For parathion and carbofuran, the amount of chemical consumed was relatively similar among concentrations producing mortality. Grue (1982) reported a similar response using grackles (*Quiscalus quiscula*) and suggested that mortality was largely due to pesticide-induced anorexia. Grue found that birds exposed to OPs, either in food or by oral gavage, significantly reduced consumption of all food, treated or untreated, for up to 12 hours. Adams (1973, 1977) and Glow *et al.* (1966) found that depression of brain ChE activity was correlated with

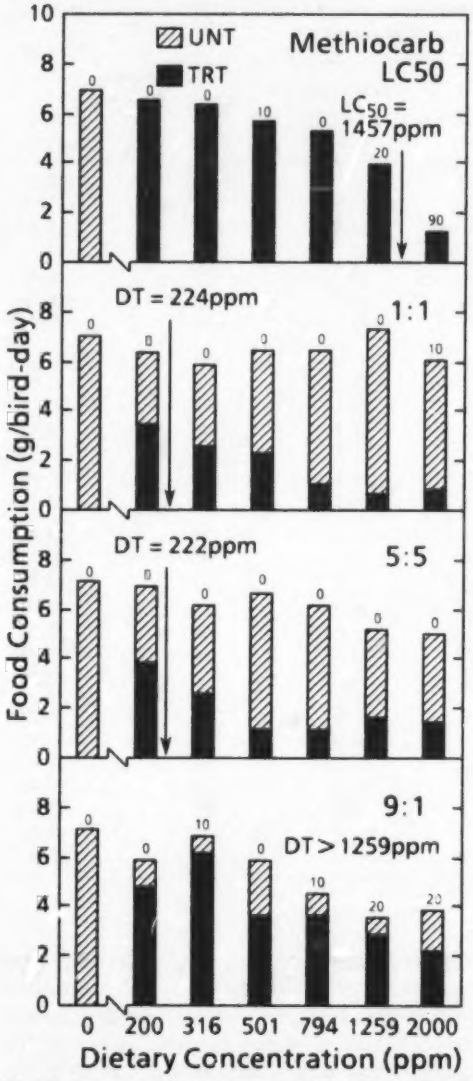

Fig. 3. Average daily food consumption for northern bobwhite presented various combinations of methiocarb-treated and untreated feeders. The values above each bar represent percent mortality during the 5-day treatment period. The discrimination threshold (DT) represents the dietary concentration above which birds show a preference for untreated food

reduced appetitive behavior and food consumption in rats, leading to loss of body weight.

Data from the present study and that from Bennett (in review) indicate that for each pesticide there is an amount of daily chemical ingestion that results in temporary cessation of feeding, whether in an LC50 or choice test. However, below that amount of chemical ingestion, birds can actively discriminate between feeders such that there is little or no reduction in total food consumption. As described above, the number and relative proportion of food choices can significantly affect whether or not birds remain below that amount.

Another important aspect to understanding dietary discrimination is the timing of formation of aversions. In the carbofuran choice tests, 50% of the mortality occurred on day 1, usually within the first few hours. Birds seemed to consume lethal quantities of chemical before any food aversion was formed. Even though many birds could discriminate between feeders after the initial exposure, the EAI based on a 5-day feeding test does not identify acute toxicity hazards.

The DT and EAI represent quantitative measures for comparing food avoidance potential among chemicals or for evaluating factors that affect this potential for each chemical. In these tests, it was observed that changes in the relative proportion of treated and untreated food sources most affected the ability of birds to reduce consumption of treated food, thereby increasing the DT and, consequently, lowering the EAI. Given the importance of this variable on the feeding responses of birds, additional information is needed to better define the conditions under which they can behaviorally respond to contaminated food by modifying their chemical ingestion. Additionally, it is not known how these responses observed in the laboratory relate to the free-ranging birds. Conover (1984) found that both caged and free-ranging birds responded similarly when methiocarb-treated and untreated seeds were presented in equal abundance. Ultimately, improving our ability to measure or predict chemical exposure will require integrating information on toxicity, behavioral responses, and the abundance and availability of less contaminated alternative foods.

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References

Adams PM (1973) The effects of cholinolytic drugs and cholinesterase blockade on deprivation based activity and appetitive behavior. Neuropharmacology 12:825–833

——— (1977) Effects of anticholinergic and cholinesterase blocking drugs on appetitive behavior under different deprivation conditions. Life Sci 21:129–136

Bennett RS (in review) Role of dietary choices on the ability of bobwhite to discriminate between organophosphate-treated and untreated food. Environ Toxicol Chem

Bennett RS, Schafer DW (in press) Procedure for evaluating the potential of birds to avoid chemically-contaminated food. Environ Toxicol Chem

Conover MR (1984) Response of birds to different types of food repellents. J Appl Ecol 21:437–443

Glow PH, Richardson A, Rose S (1966) Effects of acute and chronic inhibition of cholinesterase upon body weight, food intake, and water intake in the rat. J Comp Physiol Pschol 2:295–299

Grue CE (1982) Response of common grackles to dietary concentrations of four organophosphate pesticides. Arch Environ Contam Toxicol 11:617–626

Hill EF, Heath RG, Spann JW, Williams JD (1975) Lethal dietary toxicities of environmental pollutants to birds. U.S. Fish and Wildlife Service, Special Sci Rep—Wildl No 191, Washington, DC

Kenaga EE, Fink RJ, Beavers JB (1979) Dietary toxicity tests with mallards, simulating residue decline of chlorpyrifos and avoidance of treated food. In: Kenaga EE (ed) Avian and Mammalian Wildlife Toxicology. American Society for Testing and Materials, Philadelphia, PA, pp 36–44

Kononen DW, Hochstein JR, Ringer RK (1986) A quantitative method for evaluating avian food avoidance behavior. Environ Toxicol Chem 5:823–830

——— (1987) Avoidance behavior of mallards and northern bobwhite exposed to carbofuran-contaminated food and water. Environ Toxicol Chem 6:41–50

SAS Institute, Inc (1982) SAS User's guide: Statistics. SAS Institute, Cary, NC

Steel RGD, Torrie JH (1960) Principles and procedures of statistics. McGraw-Hill, New York

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